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Biomonitoring Studies and Susceptibility Markers for Acrolein Congeners and Allylic and Benzyl Compounds

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The importance of genotoxic acrolein congeners and allylic and benzyl compounds as industrial compounds, ubiquitous environmental pollutants, and naturally occurring substances necessitates the availability of adequate biomonitoring techniques. Endogenously formed acrolein congeners are considered to play an important role in carcinogenesis. Our studies have demonstrated that acrolein congeners react with DNA components and form adducts with the guanine moiety. We have identified and characterized cyclic 1,N^2-deoxyguanosine adducts, cyclic 7,8-guanine adducts, linear 7-guanine adducts, 1,N^2,7,8-*bis*-cyclic adducts, and 1,N^2-cyclic, 7-linear *bis* adducts. Both the reactivity of the acroleins toward nucleosides and their mutagenicity in *S. typhimurium* TA100 decrease with increasing degree of alkyl substitution. Adducts are now available as reference substances for developing sensitive detection methods. Of the biomonitoring methods investigated for allylic and benzyl compounds, the detection of cysteine and histidine adducts isolated from hemoglobin seems to be the most sensitive. Gas chromatography with electron capture detection of heptafluorobutyric acid derivatives allows a detection limit in the femtomole range, HPLC-fluorescence detection of O-phthalic dialdehyde derivatives allows a limit in the picomole range, and dectection of 9-fluorenylmethyl-chlorofomiate derivatives allows a limit in the femtomole range.

Introduction

α,β-Unsaturated carbonyl compounds (acrolein congeners) and allylic and benzylic compounds are important industrial chemicals, ubiquitous environmental pollutants, and natural products and are found in foodstuffs (*1,2*). They form DNA adducts, are genotoxic, mutagenic, and carcinogenic (*1–4*). The great importance of these compounds necessitates the availability of adequate biomonitoring techniques.

α,β-Unsaturated carbonyl compounds are also formed endogenously, e.g., during lipid peroxidation or after oxidative stress, and are considered to play an important role in human carcinogenesis (*5*). Although these endogenously formed acroleins are a constant source of DNA damage, no clear data are available for the severity of this damage and whether or to what extent these compounds lead to mutation and induce tumors in animals or humans (*5*). We have systematically studied the interaction of acrolein congeners with DNA components.

DNA Adducts with Acrolein Congeners

The detection of DNA adducts in animal or human tissue samples allows an estimation of the extent to which acrolein congeners are involved in cytotoxic and genotoxic processes and the role of these substances in mutagenesis and carcinogenesis. A first prerequisite for a specific and sensitive detection of small amounts of such adducts in human tissue is their *in vitro* isolation, identification, and characterization. Therefore, we have isolated and characterized the nucleoside and nucleotide adducts of 12 α,β-unsaturated carbonyl compounds by infrared, ultraviolet, ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and mass spectrometry as well as by melting point determination and elementary analysis. The deoxyguanosine moiety was the most reactive target in DNA. No adducts other than those with deoxyguanosine, guanine, or deoxyguanosine monophosphate were found except with acrolein, where we also found adenine adducts. All compounds (Table 1) formed cyclic 1, N^2-deoxyguanine adducts. Cyclic 1, N^2-deoxyguanosine adducts were first described for acrolein and crotonaldehyde (*6*) and for α-bromoacrolein (*7*). We can confirm these results and have identified similar adducts with 2-methyl acrolein, pentenal, hexenal, hexadienal, 3,3-dimethylacrolein, 2,3-dimethylacrolein, methylvinylketone, ethylmethyl-ketone, and α-chloroacrolein. In principle, two types of regioisomers of the 1,N^2-cyclic adducts were identified. First, one of the amino groups binds to the activated double bond (Michael addition), and then the other amino group reacts with the carbonyl carbon:

$$\underset{(R_4)(R_3)}{HCH} = \underset{(R_2)}{CH} - \underset{(R_1)}{CHO}$$

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Table 1. Isolated and characterized adducts.

Substance	1,2-Cyclic deoxyguanosine adduct IA	IB	7,8-Cyclic guanosine adduct II	7 Adduct III	*bis* Adduct IV	V
Acrolein	+	+	−	−	+	−
Crotonaldehyde	+	−	+	−	+	−
Pentenal	+	−	+	−	−	−
Hexenal	+	−	+	−	−	−
2,4-Hexadienal	+	−	+	−	−	−
3,3-Dimethylacrolein	+	−	−	−	−	−
2-Methylacrolein	+	+	−	−	−	−
2,3-Dimethylacrolein	+	−	−	−	−	−
Methyvinylketone	−	+	−	+	−	+
Ethylvinylketone	−	+	−	+	−	+
2-Choloracrolein	+	+	−	−	−	−
2-Bromoacrolein	+	+	−	−	−	−

Type A adducts are formed if the Michael addition takes place at the N2 atom of the guanine moiety. Type B adducts are formed if the Michael addition occurs at the 1-N-atom. Both types of regioisomers are formed with acrolein, 2-methylacrolein, 2-chloroacrolein, and 2-bromoacrolein. The other congeners form either type A or type B adducts but not both (Table 1), probably due to steric effects. In general, several enantiomers of each type of regioisomer were observed (Fig. 1).

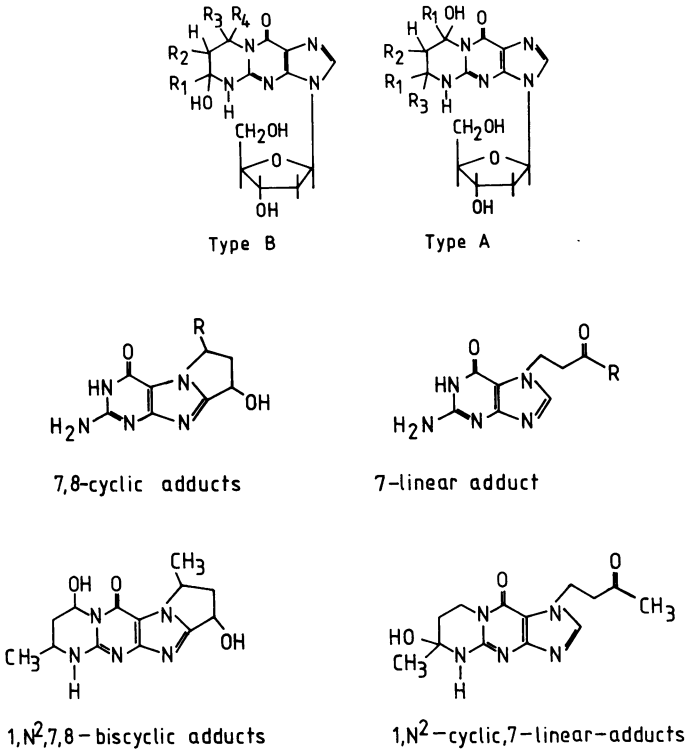
Besides the $1,N^2$-cyclic adducts, 7,8-cyclic adducts, 7-linear, $1,N^2$,7,8-*bis*-cyclic adducts, and $1,N^2$-cyclic, 7-linear *bis*-adducts were identified and characterized (Table 1; Figs. 1 and 2). More structural details and detailed characterization data will be presented in separate papers (Eder et al., manuscript in preparation). The 7,8-cyclic adduct of crotonaldehyde found in our investigations is identical to those recently found in hepatic DNA of rats treated with *N*-nitrosopyrrolidine (*8*). $1,N^2$-7,8-*bis*-cyclic adducts were first reported by Shapiro et al. (*9*) for acrolein. We found such adducts also with crotonaldehyde. Results similar to those shown for nucleosides were also obtained with nucleotides.

Reactivity and Mutagenicity of Acroleins

In general, the reactivity of the acrolein congeners toward deoxyguanosine decreases with increasing alkyl substitution. This is consistent with the mutagenicity in *S. typhimurium* strain TA100, which also decreases with increasing degree of substitution.

Biomonitoring Assay for Detecting Low-level Exposure to Allylic and Benzyl Compounds

In principle, biomonitoring can be performed by *a*) detection of typical metabolites in urine, *b*) detection of specific DNA adducts, or *c*) detection of modified amino acids from hemoglobin. Sixty to eighty percent of reactive allyl compounds are excreted as mercapturic acids (*10*). Thus, it is practicable to use gas chromatography or gas chromatography–mass spectrometry (GC–MS) detection of mercapturic acids for effective bio-



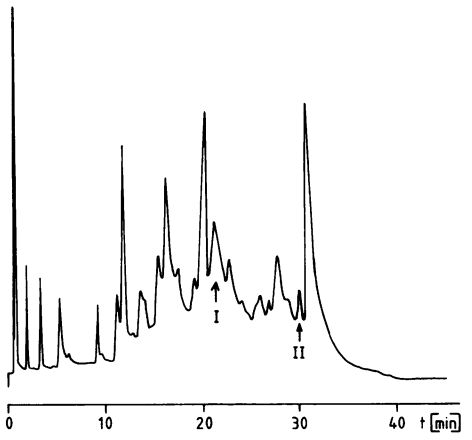


FIGURE 3. Gas chromatogram of hydrolyzed globin from benzylchloride-exposed hemoglobin derivatized with hexafluorobutyric acid with a 30 m Megabore OV-1 column (i.d. 0.53 mm) and a ^{63}Ni electron capture detection (temperature 90°C, carrier gas Argon/CH_4 95/5%). I, Benzylcysteine derivative; II, benzylhistidine derivative.

monitoring of these compounds. Allyl and benzyl compounds form direct DNA adducts (*3*), which can be used as distinctive markers for carcinogenic risk assessment.

In this paper we focus on the detecting modified amino acids from hemoglobin, which seems to be the most sensitive technique. Globin was isolated from exposed human hemoglobin and subjected to acid hydrolysis (*11*) or hydrolyzed enzymatically with pronase (Boehringer Mannheim, FRG). The benzyl cysteine and histidine adducts were either bought or synthesized as

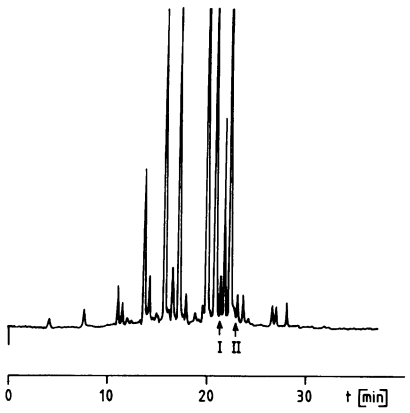


FIGURE 4. HPLC of hydrolyzed globin from exposed hemoglobin derivatized with OPA on a Lichrospher 100-RP8 (5 μm column, length 25 cm, i.d. 4 mm, flow 0.7 mL/min, gradient 100%, 12.5 M phosphate buffer, pH 7.2 to 100% methanol in 35 min). Detection was performed with a Contron Spectrofluorometer SFM 23 (excitation wavelength 326 nm, emission at 450 nm).

reference substances. Their derivatives with hexafluorobutyric acid, with OPA (*o*-phthalic dialdehyde) or with FMOC (9-fluorenylmethyl chloroformiate) were also synthesized and characterized by ^{1}H-NMR spectroscopy and mass spectrometry, and their chromatographic properties were determined either by gas-liquid chromatography (GLC) or high-pressure liquid chromatography (HPLC).

GLC and HPLC Analysis

After derivatization of the hydrolyzed hemoglobin mixture with heptafluorobutyric acid anhydride, aliquots were analyzed by GC with electron capture (ECD) detection under the conditions shown in Figure 3. The adducts were assigned by the retention times determined from the reference substances. The identity of the adducts was additionally confirmed by co-chromatography with the reference substances. The detection limit for these derivatives was in the femtomole range.

For HPLC analysis the respective adducts were derivatized either with OPA (Fig. 4) or with FMOC and assigned by retention times as well as by co-chromatography. The detection limit for the OPA derivatives was in the picomole range and that of FMOC derivatives was in the femtomole range.

Conclusions

Identication of acrolein congener adducts demonstrates that these acroleins can interact with DNA components. Well-characterized adducts are now available as reference substances for use in the development of highly sensitive techniques for the detection of trace amounts in human tissue. We are currently adapting the ^{32}P-postlabeling method using HPLC and have shown that this technique is practicable (unpublished results).

Identification of these adducts in human tissue allows a clearer estimation of the role of formed α,β-unsaturated carbonyl compounds in mutagenesis and carcinogenesis but also provides the basis for evaluation of cancer risks associated with occupational and environmental exposure. Protective measures can be taken, e.g., by providing scavengers, once the genotoxic mechanism and the extent of endogenous and exogenous exposure to compounds are known. Our studies on techniques for sensitive biomonitoring of exposure to allyl and benzyl compounds provide a basis for practicable routine methods.

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